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Effects of forest fragmentation on the morphological and genetic structure of a dispersal-limited, endangered bird species

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Abstract

Throughout the tropics, pristine forests disappear at an alarming pace. This presents a severe threat to forest-dependent species. Especially dispersal-limited understory birds are affected by forest loss. We here explored the effects of habitat fragmentation on the genetic structure and the morphology of the Ecuadorian Tapaculo (*Scytalopus robbinsi*). This bird occurs only in a small range in the premontane cloud forests of southwestern Ecuador. The global population size is declining rapidly due to habitat loss and is currently estimated at only 3000 mature individuals. We caught a total of 28 Ecuadorian Tapaculos in forests of varying size in an area of about 40 km². From each bird, we took morphological measurements and a blood sample. This was used to develop a set of 10 species-specific microsatellite primers for genetic analysis and we found that the Ecuadorian Tapaculos display high levels of genetic diversity. Additionally, we identified dispersal corridors for the species across the landscape using a least-cost path analysis. Notably, we found that wing shape is related to forest size. Individuals in smaller fragments show adaptations of the wing morphology to enhanced mobility and better flight capacity. Our results suggest that the Ecuadorian Tapaculo may rapidly adapt its morphology to the level of habitat fragmentation. This potential can possibly mitigate the risk of local extinctions of the species due to human-caused forest loss and fragmentation.

Keywords

El Oro Tapaculo, cloud forest, habitat fragmentation, wing morphology, genetic diversity, microsatellites

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Introduction

Forest loss and fragmentation are among the main drivers of species extinction in the Neotropics. For many forest-dependent species, the amount of available habitat as well as the connectivity between remaining forested patches decline. The sensitivity of a species to forest loss and fragmentation is related to the species' functional traits (Vetter et al. 2011). Especially understory insectivorous birds are sensitive to the logging of forests and therefore particularly threatened by extinction (Stratford and Stouffer 1999, Ferraz et al. 2003, Sodhi et al. 2004). Many of these species have rudimentary dispersal abilities (Moore et al. 2008) and only reluctantly cross large gaps between forest fragments (Sieving et al. 1996, Vergara and Simonetti 2006, Van Houtan et al. 2007). Even smaller distances due to valleys (Krabbe 2008) or roads (Laurance et al. 2004) can contribute to habitat fragmentation for understory birds. Migration is costly for dispersal-limited species, with the costs depending on the distance to be crossed and the mobility of the species (Tischendorf and Fahrig 2000, Moilanen and Hanski 2001). While it is assumed that the population sizes of understory birds shrink due to forest loss, habitat fragmentation makes migration between disconnected populations increasingly difficult for a high number of species.

Small populations are inherently vulnerable to genetic drift and loss of genetic diversity, which constitutes an extinction risk for populations (Frankham et al. 2002). Moreover, reduced connectivity between populations diminishes migration rates and gene flow between them (Epps et al. 2005, Coulon et al. 2006, Segelbacher et al. 2010). In case that disconnected populations are occupying different ecological environments, they may be subject to different natural selection regimes, leading to adaptive divergence of functional traits and population diversity (Hendry and Taylor 2004, Räsänen and Hendry 2008). However, the effects of gene flow and adaptive divergence as drivers of diversification in different environments are controversial (Räsänen and Hendry 2008): First, reduced gene flow may promote adaptive divergence, as it increases the independence of gene pools and the potential to diversify due to different ecological selection regimes (Langerhans et al. 2003, Hendry and Taylor 2004). Second, adaptive divergence can reduce gene flow by the evolution of reproductive isolation (Saint-Laurent et al. 2003, de León et al. 2010). Third, ongoing gene flow can favor adaptive divergence by maintaining genetic variation and non-random dispersal (Garant et al. 2005, Postma and van Noordwijk 2005). Altogether, forest fragmentation can affect the genotype by altering the levels of genetic diversity and gene flow, but can also affect the phenotype by promoting adaptive divergence in case of diverging natural selection.

Insectivorous, forest-dependent birds are particularly sensitive to the fragmentation of forests (Duncan and Blackburn 2004, Sodhi et al. 2004, Vetter et al. 2011). Heavily fragmented habitats can produce significant genetic population structuring already at a small spatial scale of less than 40 km (Moore et al. 2005, Woltmann et al. 2012). Moreover, several studies have reported changes in morphology according to the degree of fragmentation in the distribution range of a particular bird species

(Anciães and Marini 2000, Lens and van Dongen 2000, Desrochers 2010). In less fragmented forests, birds are likely to develop shorter, rounder wings than in heavily fragmented habitat, which proved advantageous for maneuvering in dense vegetation (Desrochers 2010). Longer, pointed wings enhance mobility in heavily disturbed and fragmented forests (Fiedler 2005, Desrochers 2010).

In this study, we examined the effects of forest fragmentation on the genetic and morphological structure of the Ecuadorian Tapaculo (*Scytalopus robbinsi*, Rhinocryptidae), a species almost unknown to science. This bird is endemic to the understory of cloud forests in southwestern Ecuador. In general, Tapaculos are among the species most sensitive to habitat fragmentation and are therefore considered an ideal model for assessing fragmentation effects on dispersal-limited species (Castellón and Sieving 2006). Throughout the distribution range of the Ecuadorian Tapaculo, forests are heavily degraded and fragmented, affecting the habitat of not only Tapaculos, but also of other dispersal-limited, understory species like antbirds, antpittas or hummingbirds. It is estimated that over 90% of the original forest cover in southwestern Ecuador has been logged since the beginning of the 20th century (Dodson and Gentry 1991, Best and Kessler 1995). From 2005 to 2010, the deforestation rate in Ecuador was 1.89%, which is the highest rate in South America (FAO 2010). The population size of the Ecuadorian Tapaculo thus is assumed to be declining rapidly (Krabbe and Schulenberg 1997, Hermes et al. in press) and likely the remaining populations are strongly isolated from each other, with ongoing deforestation disrupting linkages between them. The Ecuadorian Tapaculo has only limited dispersal abilities and avoids crossing areas of un-forested habitat (Krabbe and Schulenberg 1997, Hermes et al. in press). Therefore, it is possible that migration rates between different populations confined to disjunctive forest fragments are low, resulting in a clear fine-scale genetic structure, as it was shown for a similar understory bird species (Woltmann et al. 2012). In view of the high level of forest loss throughout the distribution range of the Ecuadorian Tapaculo, it is possible that individuals show morphological differences depending on the degree of fragmentation. Morphological adaptations of the flight apparatus in relation to the level of habitat fragmentation could mitigate negative effects of forest loss by improving the dispersal abilities of Ecuadorian Tapaculos and thereby maintaining population connectivity.

The ability of a species to cope with ongoing habitat fragmentation can determine its abilities to persist in a changing environment and avoid local extinction (Castellón and Sieving 2006, Stouffer et al. 2006). Therefore, we want to investigate the effects of forest fragmentation on the morphology of the Ecuadorian Tapaculo, on the level of genetic diversity and on gene flow between populations. Detailed information about these effects are crucial to make scientifically sound recommendations for conservation measures not only for this endemic species, but also for other forest specialists restricted to this kind of habitat. Given the presumably low dispersal abilities of the species and, at the same time, the high level of habitat fragmentation in the study area, we expect migration rates and gene flow between forest patches to be reduced, leading to genetically distinct sub-populations. However, not only is the species' ecology unknown, but also genetic information is lacking. We thus caught individuals to assess

the genetic status of the population. We expected to find genetic differentiation between individuals caught in locations separated by dispersal barriers, like areas of open habitat, unsuitable elevation, or highways. To identify corridors with low dispersal cost, i.e., the optimal routes for migration of Ecuadorian Tapaculos, we calculated least-cost paths between territories. Moreover, we assessed morphological differences of birds caught in different sites of the study area; we predicted to find differences according to the level of habitat fragmentation.

Methods

Study species and study area

The Ecuadorian Tapaculo, also known as El Oro Tapaculo, is an insectivorous bird endemic to a small range (~ 1100 km^2) on the western slopes of the Andes in southwestern Ecuador, at an elevation of 850–1500 m (Hermes et al. in press). The species was only discovered in 1990 (Krabbe and Schulenberg 1997) and is so far not well studied. It occurs in the undergrowth of mature forests and is very reluctant to cross even small areas of open habitat (Krabbe and Schulenberg 1997). Being practically unable to fly longer distances, Tapaculos move around by hopping or walking (Reid et al. 2004, Castellón and Sieving 2006). The IUCN classifies the Ecuadorian Tapaculo as endangered. Global population size is estimated to range between 1900 and 4600 mature individuals (Hermes et al. in press). It is feared that the species' requirements for high-quality habitat and presumed susceptibility to forest degradation and fragmentation have led to a severe population decline, which might still be ongoing (Hermes et al. in press).

The only protected site within the range of the Ecuadorian Tapaculo is the private Buenaventura reserve in the canton Piñas (3.655°S, 79.744°W), established in 1999 by the Ecuadorian NGO Fundación Jocotoco. This reserve covers an area of 2300 ha in an elevation of 400–1500 m (Figure 1). The predominant vegetation types within the reserve are secondary forests in various successional stages, which are separated by areas of abandoned pasture. Outside the reserve, deforestation is intense, with mostly only forest patches smaller than 100 ha remaining. The main causes for the logging of forests are intensification of agriculture and forest clearance for livestock. Natural forests mainly persist in areas which are not suitable for conversion into cattle pasture or cropland, like steep slopes or river banks (Best and Kessler 1995).

Field work was carried out between December 2013 and May 2014 and between November 2014 and January 2015 in the Buenaventura reserve, and near Ñalacapa, about 5 km south of Buenaventura (Figure 1). The study area is located at the southern end of the Ecuadorian Tapaculo's distribution range, covering about 5% of the total range. The size of forest fragments was assessed in ARCMAP 10.2 using satellite images of the area as a template (Hermes et al. in press). Forest areas ranged from about 15 ha to 900 ha. The northern and southern part of the study area was divided by a highway and a valley with an altitude of about 400 m.

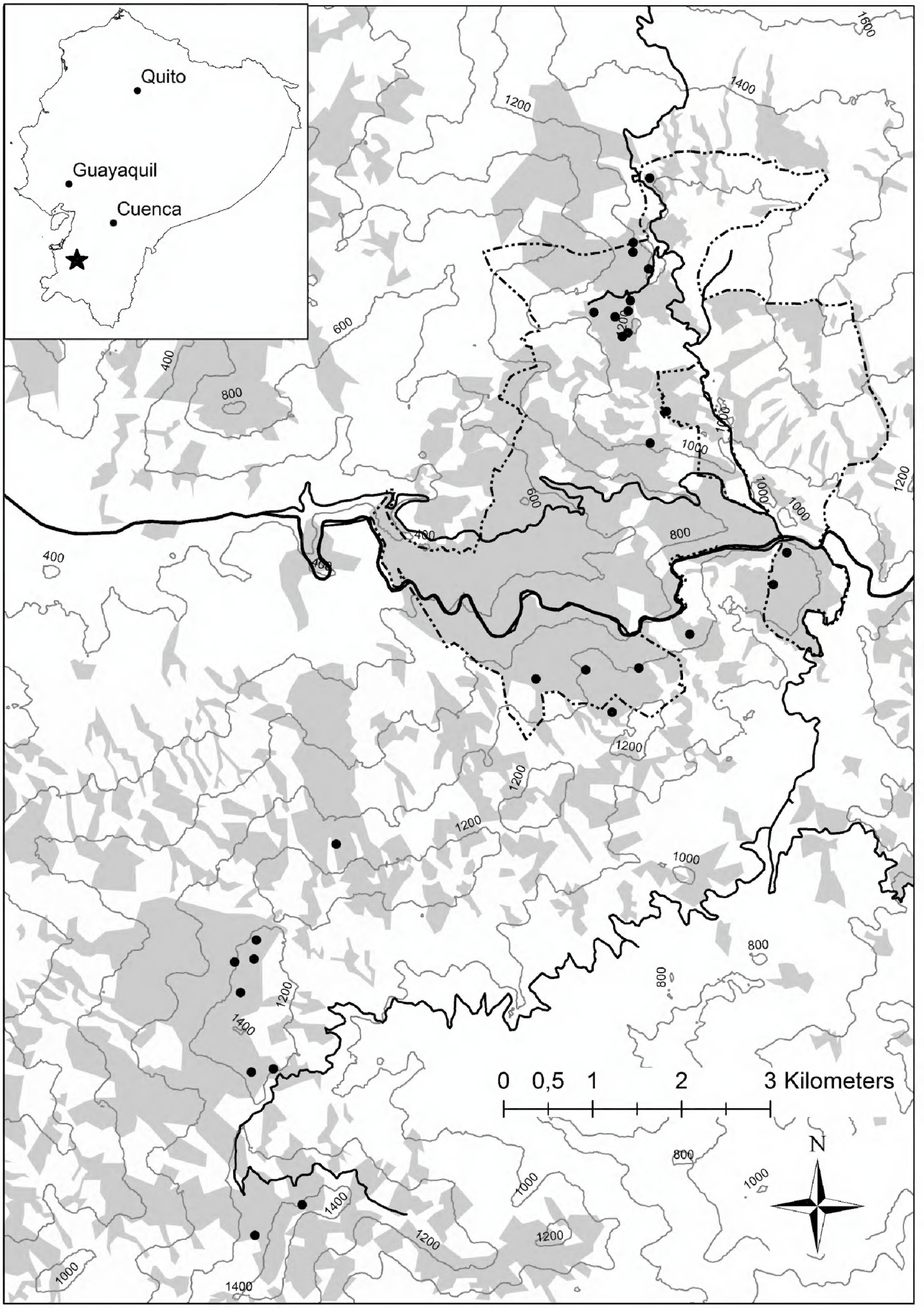

Figure 1. Map of the study area in southwestern Ecuador. Forested areas are shaded grey, whereas white areas represent non-forested areas (mainly cow pastures). The Buenaventura reserve is circled by the dashed line. The bolt black line represents a highway cutting the reserve into a northern and a southern part, while minor roads are indicated by the thin lines.

Bird sampling

For bird capturing, we used mist-nets and tape recordings of the song of male Ecuadorian Tapaculos as a decoy. If an individual approached the playback, observers herded it into the net. The Ecuadorian Tapaculo's secretive behavior, very good vision and excellent maneuverability made the capturing very challenging. We captured 28 males. Birds were ringed with a standard aluminum ring and color-banded individually. Then, individuals were weighed and the lengths of tail, tarsus, wing, primary feathers and the first secondary feather were measured. From each individual, we took a blood sample from the brachial vein. To minimize stress, birds were handled within less than 10 minutes of capture and released unharmed to the same sites. Blood samples were stored in 99.8% ethanol and transferred into a -20 °C freezer.

Assessment of morphological differences

To obtain an index for body size, we carried out a principal component analysis (PCA) for all the morphological variables that we recorded. As variables differed in their numerical range, they were z-standardized prior to the analysis. A second PCA for the variables wing length and length of the feathers P9 to S1 provided an index for the wing shape. For both PCAs, missing values (e.g., caused by feather molt) were replaced by the mean. Additionally, we quantified the body condition of each individual using the scaled mass index (Peig and Green 2009). Then, we tested for relationships between an individual's morphology and the size of the forest where it was captured. To this aim, we carried out Kendall correlations between the fragment area and the first principal components of the PCAs for body size and wing shape as well as the body condition index. The statistical analysis was carried out in R 3.3.0 (R Development Core Team).

Analysis of genetic population structure

We extracted DNA from the blood samples and compiled a set of 10 species-specific microsatellite primers (for a description of the primer development see Supplementary File). Two individuals had to be excluded from the analysis due to failure of amplification during PCR in two loci. Then, we applied a Bayesian clustering method using the program STRUCTURE 2.3.4 (Pritchard et al. 2000) to explore the genetic population structure of the individuals (n = 26) caught in different fragments. This program uses a Markov chain Monte-Carlo (MCMC) approach to compute the probability of the sampled individuals belonging to a given number *K* of discrete genetic subpopulations. An admixture model with correlated allele frequencies was used. We pre-defined the parts north and south of the highway as distinct sampling locations (Figure 1).

We set *K* from 1 to 8 and carried out 10 runs for each *K*, with 10^6 MCMC iterations and 500,000 burn-in iterations for each run. We determined the best value for *K* by analyzing the probability scores in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Least-cost paths and isolation by distance

Landscape barriers disrupting or decreasing connectivity between individuals or populations can be quantified and qualified by the creation of a resistance map, which allocates a specific resistance value to each cell of the land cover grid according to the mobility of the species (Adriaensen et al. 2003). To evaluate landscape permeability for Ecuadorian Tapaculos in the study area and to assess least-cost paths (LCPs) between the individuals, we created a resistance map accounting for species-specific demands and landscape features. We produced a map (cell size 30 x 30 m) for the parameters 'forest cover', 'elevation', and 'roads' by assigning them different weights in the RASTER CALCULATOR tool of ARCMAP. Weights were determined on expert-opinion based on literature review and observations of the behavior of individuals. As Ecuadorian Tapaculos are reluctant to cross open habitats without forests (Krabbe and Schulenberg 1997), we assumed the costs for crossing open areas to be 100-fold higher than for dispersing through forests. Roads are known to represent a strong dispersal barrier for understory birds; even narrow, unpaved roads significantly reduce dispersal, while highways can even entirely block movement (Laurance et al. 2004). We created a buffer zone with a radius of 15 m around the roads in the study area in order to obtain a continuous reproduction of the roads on the 30 × 30 m resolution of the map. We assigned a 200-fold weight to the highway dissecting the northern and southern part of the study area, while the less frequented country lanes only obtained a 100-fold weight. For the resistance values of the elevation, we considered the mean altitude of territories to be the optimum for Ecuadorian Tapaculos, with dispersal costs being zero. There are hints that the Ecuadorian Tapaculo is sensitive to elevation. Presumably, the species has shifted its distribution range uphill within the last decades and now avoids areas of lower elevation (Hermes et al. in press). Therefore, higher or lower elevations were assigned costs equaling the difference in altitude to the mean altitude of territories. Merging of the layers generated the resistance map. The LINKAGE MAPPER tool of ARCMAP was then used to identify the LCP between territories by detecting cells with the lowest costs while avoiding cost-intensive cells.

To assess isolation by distance, we tested for relationships between the genetic and the geographic distances between the individuals. Geographic distance was expressed by Euclidian distance between territories as well as by LCP length and LCP cost. In GENALEX 6.5 (Peakall and Smouse 2012), we carried out Mantel tests with 999 permutations for each of the three parameters separately.

Analysis of past genetic diversity and population size change

Using the R package HIERFSTAT, we tested whether genetic diversity has been reduced since the species was discovered in 1990. Accounting for the difference in sample size between the two groups, we compared allelic richness of the 26 samples we took in 2013–2015 with those of seven museum specimen collected in 1990–1991 in the same area (from the tissue collection at the Zoological Museum Copenhagen; sample numbers 125057, 125070, 125071, 125072, 126057, 126058 and 126167).

Additionally, we tested for a potential decline in the effective population size in the past with the program MSVAR 1.3 (Beaumont 1999, Storz and Beaumont 2002). This program was shown to be particularly powerful at detecting severe and ancient population declines (Girod et al. 2011), and can deal with small sample and population sizes (Beaumont 1999). Using multilocus microsatellite data, MSVAR applies a Bayesian coalescent-based hierarchical model to estimate the current population size as well as the ancestral population size, the time since a potential population decline or expansion started and the mutation rate of loci. With MCMC simulations, the program quantifies the likelihood of observing the allele frequencies in a sample, given a pre-defined demographic and mutational model. The simulation then produces probability estimates for the above-mentioned parameters by maximizing the likelihood of the observed data. We ran the model four times. To avoid a bias on the posterior distribution, each time we used different prior information assuming different scenarios of past population size change. We ran each chain with 10^9 iterations and a thinning interval of 100,000. Thus, we obtained an output of 20,000 iterations for each run and dismissed the first 5,000 iterations as burn-in. The output was analyzed using the R packages CODA, BOA and LOCFIT. We checked the output chains for convergence using the Gelman-Rubin analysis (Gelman and Hill 2007) and calculated modes and 95% highest probability density (HPD) intervals for each parameter. Parts of the R script were taken from Paz-Vinas et al. 2013.

Results

Bird morphology in relation to forest size

The first four principal components (PCs) of the PCA of body size accounted for 71.92% of the variance of 14 morphological variables (Table 1). We assigned loadings above a threshold of 0.35 to the respective PC. PC 1 described the length of the inner primary feathers P5, P3, P2 and P1, while PC 2 described the outer primary feathers P9, P8 and P7. PC 3 characterized the length of tarsus and the P4 feather, and PC 4 described the length of wing and tail. The PCA for the wing shape yielded similar results, with the first three PCs accounting for a total of 74.82% of the variance (Table 2). Here, PC 1 also represented the primary feathers P5, P3, P2 and P1. PC 2, accordingly, described the feathers P9, P8 and P7. PC 3 characterized the total

Table 1. Body size of Ecuadorian Tapaculos. Principal component analysis for the body size of 28 Ecuadorian Tapaculos, with the loadings, eigenvalues and variance of the first four principal components (threshold: 0.35; bold font).

Variables	Loadings			
	PC 1	PC 2	PC 3	PC 4
Tarsus	-0.037	-0.052	**0.641**	0.087
Wing	-0.170	0.001	-0.326	**0.549**
P9 feather	-0.187	**-0.534**	-0.185	-0.173
P8 feather	-0.269	**-0.398**	-0.024	-0.045
P7 feather	-0.270	**-0.356**	0.102	-0.190
P6 feather	-0.321	-0.258	-0.077	0.029
P5 feather	**-0.356**	0.102	-0.180	0.327
P4 feather	-0.203	0.193	**-0.355**	-0.193
P3 feather	**-0.388**	0.202	0.041	-0.003
P2 feather	**-0.373**	0.238	0.041	0.067
P1 feather	**-0.371**	0.189	0.240	-0.069
S1 feather	-0.300	0.235	0.317	-0.192
Weight	0.002	-0.339	0.267	0.174
Tail	0.043	-0.095	0.205	**0.634**
Eigenvalue	5.202	1.889	1.627	1.351
Variance explained	37.16%	13.49%	11.62%	9.65%

Table 2. Wing shape of Ecuadorian Tapaculos. Principal component analysis for the wing shape of 28 Ecuadorian Tapaculos, with the loadings, eigenvalues and variance of the first three principal components (threshold: 0.35; bold font).

Variables	Loadings		
	PC 1	PC 2	PC 3
Wing	-0.174	0.004	**0.669**
P9 feather	-0.187	**-0.593**	0.050
P8 feather	-0.270	**-0.401**	-0.047
P7 feather	-0.270	**-0.363**	-0.346
P6 feather	-0.323	-0.292	0.042
P5 feather	**-0.359**	0.106	**0.379**
P4 feather	-0.205	0.126	0.167
P3 feather	**-0.388**	0.207	-0.050
P2 feather	**-0.372**	0.261	0.082
P1 feather	**-0.359**	0.230	-0.224
S1 feather	-0.300	0.284	**-0.442**
Eigenvalue	5.188	1.787	1.256
Variance explained	47.16%	16.25%	11.42%

wing length, as well as the length of the feathers P5 and S1. We concluded that, for both PCAs, individuals with high values for PC 1 have shorter inner primaries, i.e., a narrow wing, and individuals with high values for PC 2 have shorter outer primaries, i.e., a less pointed wing.

For both PCAs, we detected a marginally significant relationship between PC 1 and forest size (PCA of body size: P = 0.057; tau = -0.273; and PCA of wing shape: P = 0.063; tau = -0.267; Kendall correlation). None of the other PCs correlated with forest size (all P > 0.12; Kendall correlation). Similarly, there was no relationship between the body condition of birds and the size of the forest fragments (P = 0.76; tau = 0.044; Kendall correlation).

Genetic diversity, population genetic structure and gene flow

Allelic richness of the museum samples was 3.80 ± 0.75, while that of the recently collected samples was 3.59 ± 0.57. Therefore we concluded that genetic diversity has not changed within the last ~ 25 years. The STRUCTURE analysis showed no clear population substructure. $K = 1$ yielded the highest probability, indicating that most likely all samples belonged to the same population. However, error bars were highly overlapping amongst the estimates for different numbers of clusters (Figure 2).

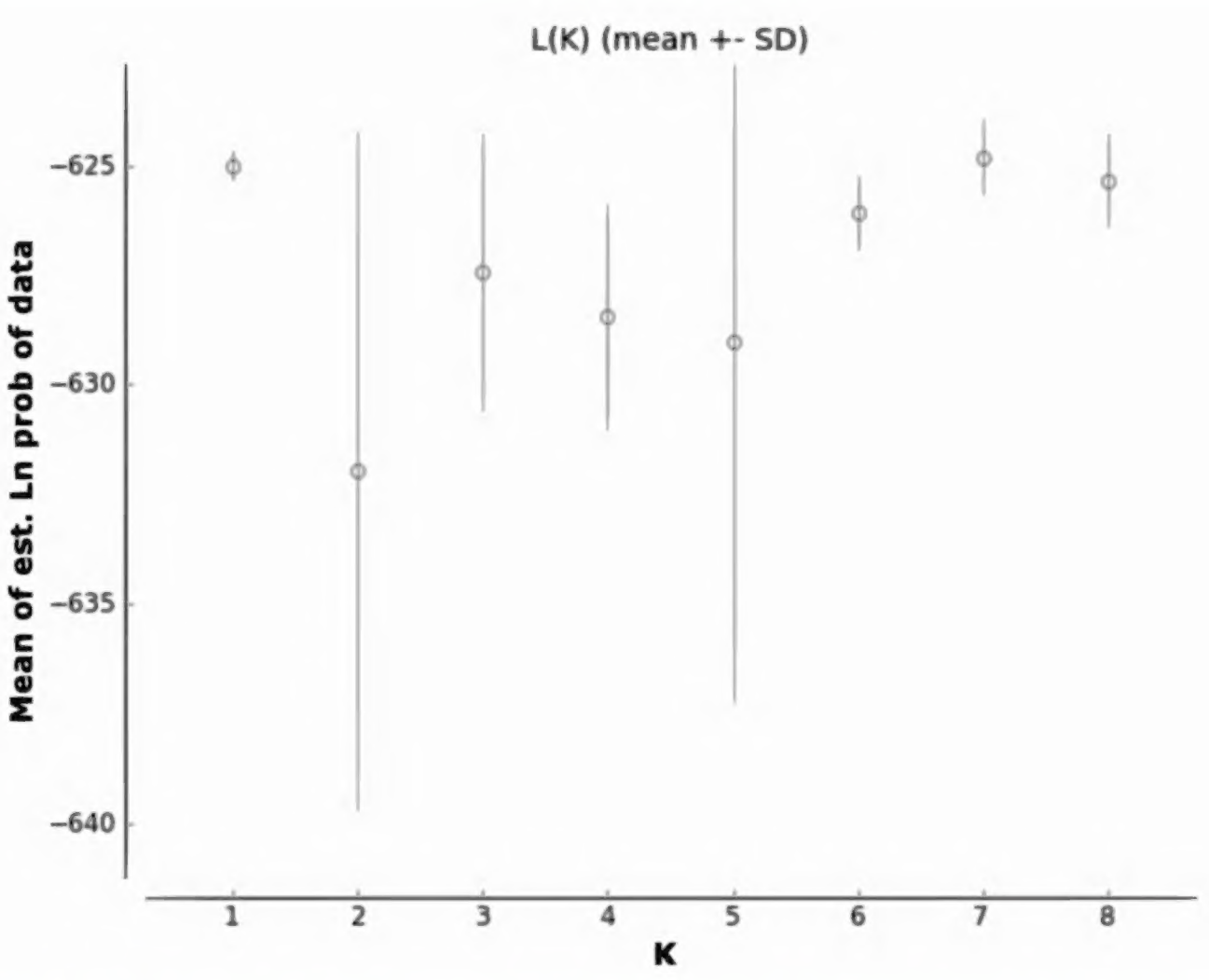

Figure 2. Mean ± SD of the log-likelihood for $K = 1$ to 8 distinct genetic populations. Strong support for $K = 1$ indicates that most likely all the samples stem from the same genetic group.

With the least-cost path analysis, we could identify a dispersal corridor for Ecuadorian Tapaculos across the study area, which circumvented the valley between the northern and southern part (Figure 3). Mantel tests indicated clear evidence for isolation by distance. Euclidian distance showed the strongest relationship to the genetic distance (R_{xy} = 0.418; P = 0.001), followed by LCP length (R_{xy} = 0.399; P = 0.001) and LCP cost (R_{xy} = 0.319; P = 0.001).

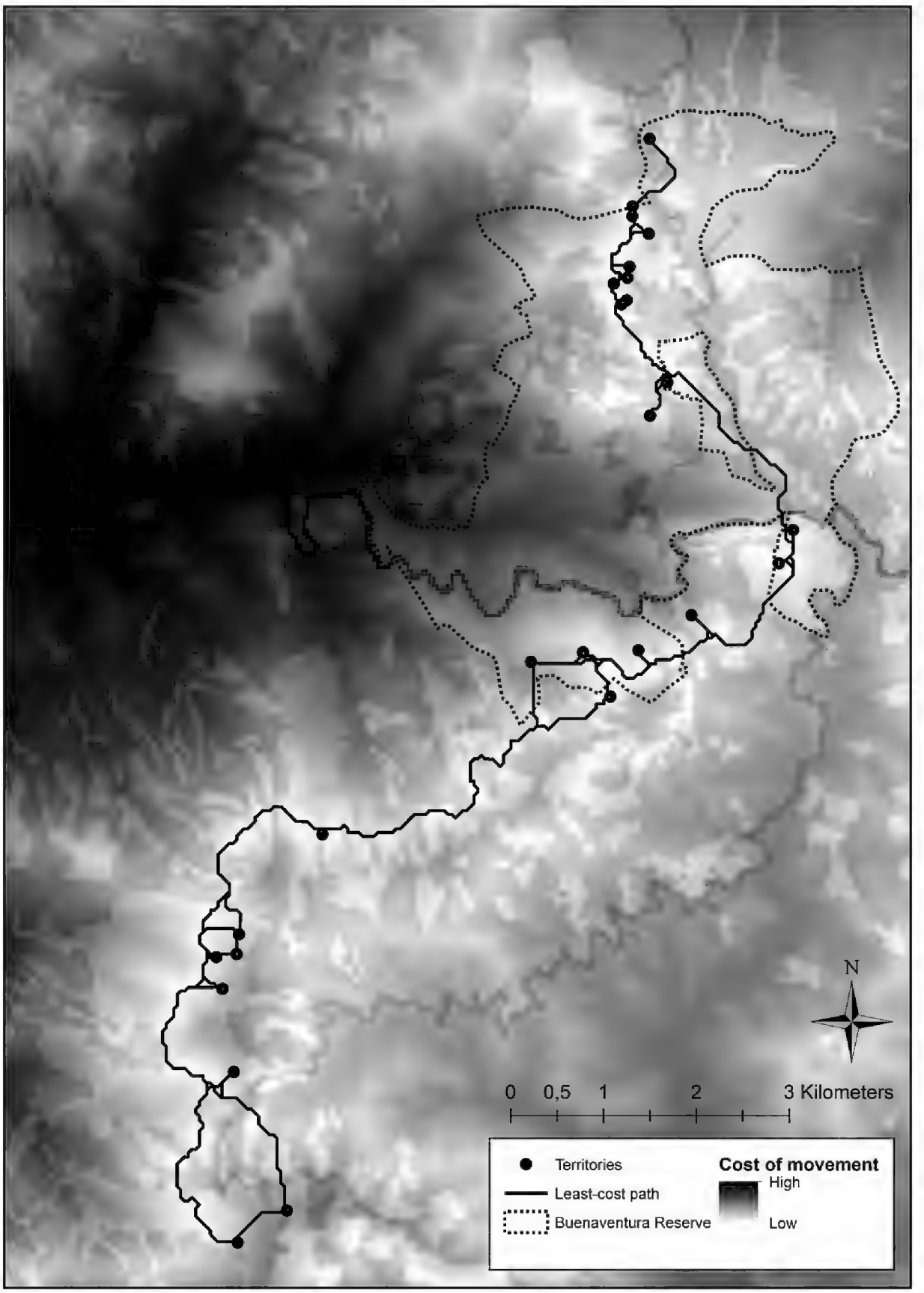

Figure 3. Resistance map with least-cost path. The cost of movement is visualized by the color gradient from black to white, with black indicating higher costs and white lower costs. The bolt black lines show the least-cost paths between 26 Ecuadorian Tapaculo territories in the study area.

Past demographic changes

Modelling the population demography yielded evidence of a severe population decline in the past. All potential scale reduction factors were < 1.1, so we concluded that chains converged well (Gelman and Hill 2007). Modal values (and 95% HPD intervals) indicated a current effective population size of 770 individuals (150–2,820). Ancestral population size was 26,000 (5,275–171,400), suggesting an approximately 30-fold population decline. Time since the population started decreasing was estimated to about 7000 years (870–52,000) and the mutation rate to $1.42e^{-4}$ ($1.32e^{-6}$–$7.23e^{-3}$). However, the large probability density intervals for the parameter estimates indicate a high level of uncertainty in the simulation.

Discussion

In this study, we investigated the genetic and morphological structure of the Ecuadorian Tapaculo, an endangered bird endemic to the understory of premontane cloud forests in southwestern Ecuador. In the study population, genetic diversity has remained constant within the last 25 years, even though the global population has declined dramatically. Despite the fact that forests are highly fragmented and the species has only limited dispersal abilities, we did not detect a structuring into genetically distinct sub-populations on a scale of 40 km^2. Notably forest size influenced bird morphology, with individuals in larger fragments having rounder wings than their conspecifics in smaller forests.

Population genetics of the Ecuadorian Tapaculo

Throughout the study area, we found no genetic structuring among Ecuadorian Tapaculos, indicating that a substantial amount of gene flow is still maintained. Even though the Ecuadorian Tapaculo is a bad disperser, migration between different forest fragments seems not to be blocked. We expected that habitat fragmentation in the range of the species produced genetically distinct populations in different forest fragments, as it was shown for a similar species (Woltmann et al. 2012). Our study area was disrupted by a valley and a highway, which we expected to act as barriers to dispersal. Besides, the distances between the different forest fragments following the least-cost path ranged between 10 m and 400 m and were thus partly larger than the mean dispersal distances observed for Ecuadorian Tapaculos (80 m; Hermes et al. in press). Nevertheless, we did not detect genetic structuring in distinct sub-populations. Similarly, a study analyzing genetic differentiation in White-ruffed Manakins (*Corapipo altera*) at a comparable scale than our study did not detect genetic structuring either, although the habitat was highly fragmented (Barnett et al. 2008). However, fragmentation does not necessarily lead to reduced gene flow and genetic differentiation (Galbusera et al. 2004). Even a species with strong dispersal limitation can show low levels of differentiation across a

highly fragmented landscape (Callens et al. 2011). In the case of the Ecuadorian Tapaculo, even the high degree of forest fragmentation in the northern part of the study area is not sufficient to cause genetic structuring.

Gene flow across the study area is not impeded by barriers and Mantel-tests between genetic and geographic distances suggest isolation by distance (IBD). In theory, IBD can lead to considerable genetic differentiation even at small scales (Wright 1943). In the most extreme dispersal event observed in the Ecuadorian Tapaculo, an individual crossed 245 m of un-forested habitat to establish a territory in a remote forest fragment (Hermes et al. in press). The IBD detected here is therefore likely a consequence of the generally low dispersal capacities and mean migration distances of the species, which are reinforced by forest fragmentation.

Genetic diversity of Ecuadorian Tapaculos in the study area remained constant between 1990 and 2015. On a global scale however, population size and most likely also genetic diversity still decrease. Even though the result of the analysis of past population demography yielded a high level of uncertainty and should therefore be treated with caution, it gave evidence of a severe population decline. The Buenaventura reserve remains until now the only protected site within the distribution range of the Ecuadorian Tapaculo. Around the reserve, forests are heavily fragmented and degraded; mostly, patches are smaller than 100 ha and consist of young secondary stands. The constant level of genetic diversity in the study population over 25 years, which is presumably attributable to the establishment of the reserve, shows that a negative population trend can be stopped. However, in order to achieve a change for the better on the scale of the global population of Ecuadorian Tapaculos, it would be necessary to protect remaining forests throughout the entire distribution range, which, in view of ongoing deforestation, seems implausible. In general, Tapaculos are among the understory species most sensitive to fragmentation and are therefore seen as umbrella species for conservation planning (Willson et al. 1994, Reid et al. 2002, Castellón and Sieving 2007). The fact that we found population connectivity and a constant high level of genetic diversity in the Ecuadorian Tapaculo gives hope that other understory birds and dispersal-limited mammals in the area show similar population trends.

Morphological adaptations to forest fragmentation

While several studies have already addressed the effects of forest fragmentation on the genetic structure of a population, its effects on individual morphology are far less examined. However, the degree of habitat fragmentation can cause different morphological adaptations in birds (Desrochers 2010). Increasing distance between forest fragments exerts a selective pressure for enhanced mobility and flight ability, i.e., more pointed wings, in order to enable migration between remote fragments (Fahrig 2003, Fiedler 2005, Desrochers 2010). While the studies of Fiedler (2005) and Desrochers (2010) were carried out at a much larger spatial scale (several 1000 km), we found effects on wing morphology already at a distance of less than 15 km.

Ecuadorian Tapaculos have short, round wings and only limited flight capacities; they do rarely fly distances longer than 3 m and move mainly by walking or hopping (Krabbe and Schulenberg 1997). In this study, we found wing shape to be related to forest size. Individuals in small patches had narrow wings, which can be seen as an adaptation to enhanced mobility and better flight capacity, which probably allowed colonization in the first place. In larger fragments, on the other hand, selection pressure for increased mobility is absent. Dispersing individuals do not face the necessity to cross habitat gaps before establishing their territories. For movement within large fragments, round wings enabling good maneuverability are advantageous. Alternatively, the differences in wing shape could be caused by different structural characteristics of the understory layer in relation to fragment size, with pointier wings facilitating flights in search for food. However, microhabitat structure of the understory in the forest fragments was assessed in a previous study (Hermes et al. in press), but had no influence on wing shape. Therefore, we conclude that the morphological differences are most likely caused by the fragmentation of forests and not by the degradation within forests.

Wing morphology is highly heritable in birds (Boag and van Noordwijk 1987). In this study, we detected effects of forest fragmentation on the morphology of the species already in a small population and at a small spatial scale. This implies that habitat fragmentation exerts considerable selective pressure favoring adaptive divergence of wing morphology. However, the morphological variability of the Ecuadorian Tapaculo gives evidence of the species' potential to rapidly adapt to environmental changes. This potential can possibly mitigate the risk of local extinction of the Ecuadorian Tapaculo due to human-caused forest loss and fragmentation.

In the study population, phenotypic divergence in wing shape could arise in sympatry. Even though the individuals in the study area were not genetically differentiated at neutral markers, the morphological changes are likely promoted by the isolation by distance we discovered over the study area. Moreover, the differences in the level of forest fragmentation likely exert a selective pressure, which is strong enough to produce distinct phenotypes despite the homogenizing effect of gene flow. If the diverging selective pressures are high, a new beneficial allele can fix quickly and affect the genome (Crisci et al. 2016). In the case of the Ecuadorian Tapaculo, morphological adaptations have possibly arisen rapidly after the onset of intense forest fragmentation at the beginning of the 20th century. Similar to our results, a study on Wedge-billed Woodcreepers (*Glyphorynchus spirurus*) found considerable morphological differences, although the level of gene flow was high (Milá et al. 2009). Generally, gene flow is assumed to constrain adaptive divergence by homogenizing the gene pool (Hendry and Taylor 2004, Räsänen and Hendry 2008). However, adaptive divergence caused by environmental differences can also constrain gene flow by the evolution of reproductive isolation over a few generations, i.e., ecological speciation (Schluter 2000, Carroll et al. 2007, Hendry et al. 2007). Moreover, in case that the adaptive divergence reduces the fitness of migrants between different environments, a negative feedback loop

can be initiated: Reduced fitness of migrants reduces dispersal between the different environments, which in turn reduces gene flow. This can lead to a further increase in adaptive divergence and a further reduction in dispersal and gene flow (Räsänen and Hendry 2008). It is possible that the Ecuadorian Tapaculos are currently beginning a similar loop. Their flight apparatus is adapted to the specific level of habitat fragmentation and can be disadvantageous in different conditions. Therefore, the fitness of birds migrating to forests with a differing degree of fragmentation is likely reduced. Even though gene flow is not diminished at present, it is possible that it will decrease in future under ongoing diverging selection, forming genetically and morphologically distinct sub-populations. Nevertheless, this is not the only possible future scenario. Throughout the study area, considerable reforestation efforts have been made within the last 20 years. Forest regrowth increases habitat availability and homogeneity for the Ecuadorian Tapaculo. Thus, assuming far-reaching reforestation programs, the selective pressure for adaptations to enhanced mobility might disappear, reducing the divergence in wing morphology and increasing gene flow.

Compliance with ethical standards

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Permissions to conduct field work (No. 005-IC-FAN-DPEO-MAE) and to export samples (No. 05-2014-FAU-DPAP-MA) were granted by Ministerio de Ambiente and Ministerio de Agricultura, Ganadería, Acuacultura y Pesca, Ecuador.

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Supplementary material 1

Development of species-specific microsatellite primers

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Data type: Adobe PDF file

Explanation note: The supplementary material contains a detailed description of the development of a set of 10 microsatellite primers for the Ecuadorian Tapaculo, including primer sequences and gene bank accession numbers.

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